Asymptomatic brucellosis infection in humans: implications for diagnosis and prevention

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Abstract

Human brucellosis is mainly caused by contact with *Brucella*-infected animals and their secretions and carcasses. Individuals who are continuously in contact with animals are considered to be at a high risk but only some show symptoms and are diagnosed as cases of brucellosis. Here, we showed that asymptomatic brucellosis infections occur among humans. Asymptomatic infections mainly result from less frequent contact with *Brucella* and/or contact with low-virulence *Brucella*. In our study, patients with asymptomatic infection had low antibody titres and different contact patterns. Awareness of asymptomatic infection is important for early diagnosis of brucellosis and prevention of chronic infection.

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Brucellosis is a common zoonosis caused by *Brucella* spp. In humans, it is caused by ingestion of raw dairy products, consumption of infected meat from domestic livestock, and close contact with their secretions and carcasses [1]. High fever, myalgia and arthralgia of the large joints are the main symptoms. In brucellosis-endemic areas, individuals who are continuously in contact with animals are at a high risk of becoming infected [2]. However, only some manifest clinical symptoms, visit clinics and are diagnosed with brucellosis. In cases of infectious disease, the absence of clinical symptoms can be due to either true non-carrier status or asymptomatic infection [3]. In asymptomatic infection and subclinical infection, individuals carry the disease without symptoms, but will be symptomatic under some conditions, such as decline in immune status or re-infection. Awareness of asymptomatic infection is important for timely diagnosis and effective treatment of infectious diseases. Asymptomatic infection was observed in occasionally infected patients [4]. However, it is not known whether some high-risk individuals without clinical symptoms of brucellosis are actually infected, and this needs to be established.

To test the possibility of asymptomatic brucellosis infection in humans, we firstly recruited high-risk individuals from Jilin Province, a region of endemic human brucellosis infection in China [5]. The participants all had a history of continuous contact with animals and/or their products, had not been immunized with *Brucella* vaccines previously, and did not show the typical symptoms (continuous fever, sweating, fatigue and arthralgia) of brucellosis. Serum samples were collected and tested for *Brucella* antibodies using the Standard Agglutination Test (SAT). Serum samples were diluted at 1:50, 1:100 and 1:200 and tested with SAT. SAT titres $\geq$ 1:100 were defined as positive. The results showed that approximately 45% (135 of 300) of individuals tested positive for *Brucella* antibodies. To further test the existence of *Brucella*, blood samples were collected from antibody-positive participants and *Brucella* DNA was detected by an in-house real-time PCR (forward primer, GCCAATGCCATTGCCAAG; reverse primer, GTTCACT GATTTCCACCACAC; Taqman probe, FAM-AAGACGC TTGCCGACGCTGC-TAMRA) developed essentially as described previously [6,7]. The real-time PCR showed a sensitivity of 10 copies per reaction. *Brucella* DNA was detected in 25% (25 of 100) of the blood samples. These data implied that asymptomatic infection exists in high-risk individuals. In practice, we have found that some patients with a long history of animal contact had chronic infection when diagnosed. Long-term treatment is necessary for these patients [8]. The presence of *Brucella* DNA in blood samples of these patients implied that they were in active infection or recovery stages. This was observed in a previous study, which found that *Brucella* DNA could be detected from months to 2 years after recovery from symptoms [9].

Next, information on asymptomatic and symptomatic individuals was collected and analyzed to further characterize asymptomatic infection and differentiate it from symptomatic infection. As shown in Table 1, symptomatic patients mainly had the following clinical symptoms: fever (78.0%), sweating (41.3%), weakness (37.7%) and headache (26.1%). Symptomatic patients usually had more than one typical symptom. In the asymptomatic group, all the individuals showed none of the symptoms. More men were symptomatic than women, and symptomatic patients were older than asymptomatic patients, but this latter result was not significant. For these symptomatic patients, the average duration from first appearance to diagnosis was 62.3 (95% CI, 47.8–76.8) days. Antibody titres were tested by SAT and compared between the two groups. The titres of asymptomatic patients were significantly lower than those of symptomatic patients (116.7 vs. 143.5, $p < 0.05$). A high proportion of the asymptomatic patients had SAT titres of 1:100, indicating that asymptomatic infection patients had low antibody titers. This lower antibody titre in asymptomatic patients might result from lower frequency or longer duration of infection.

Animal contact is the main risk factor for human brucellosis [10]. Asymptomatic patients had a slightly lower frequency of sheep contact but higher frequencies of cattle contact (15.9% vs. 8.3%, p 0.013) and pig contact (19.2% vs. 8.5%, p 0.001) than symptomatic patients. No significant differences were observed in contact frequencies with goats, dogs or deer, which seems to be consistent with the different virulence of various *Brucella* species epidemic in this region. According to host preference, *Brucella* is conventionally divided into six different species, including *Brucella melitensis* (sheep), *B. abortus* (cattle), *B. canis* (dogs), *B. suis* (pigs), *B. ovis* (goats) and *B. neotome* (desert and wood rats) [11]. Four of these species are pathogenic in humans, with *B. melitensis* being the most virulent species, followed by *B. abortus*, *B. suis* and *B. canis*. In Jilin province, the most predominant *Brucella* species are *B. melitensis* followed by *B. abortus*, and other species are seldom identified. Therefore, the differential animal contact frequencies between participants with asymptomatic infection and those with symptomatic infection are consistent with the fact that *B. abortus* (cattle) and *B. suis* (pig) have lower virulence in humans than *B. melitensis* (sheep) [12].

TABLE 1. Comparison of asymptomatic and symptomatic infection

Variables	Asymptomatic (*n* = 131)	Symptomatic (*n* = 506)	p value (95% CI)
Demographics			
Male	97 (74.0%)	395 (77.4%)	0.355 (0.346–0.365)
Age	41.85 (39.48–44.22)	42.84 (41.62–44.05)	0.365 (0.294–0.398)
Contact animal			
Sheep	53 (42.4%)	243 (48.2%)	0.273 (0.264–0.281)
Goat	3 (2.4%)	11 (2.2%)	1.000 (1.000–1.000)
Pig	24 (19.2%)	43 (8.5%)	0.001 (0.001–0.002)
Dog	39 (31.2%)	123 (24.4%)	0.139 (0.132–0.145)
Deer	2 (1.6%)	3 (0.6%)	0.261 (0.2524–0.269)
Cold Sheep	33 (26.4%)	155 (30.8%)	0.385 (0.375–0.394)
Cattle	20 (15.9%)	42 (8.3%)	0.013 (0.011–0.016)
Contact manner			
Skin	103 (78.6%)	395 (78.1%)	0.904 (0.898–0.909)
Fur	82 (62.6%)	357 (70.6%)	0.087 (0.082–0.093)
Abortion	53 (40.5%)	263 (52.0%)	0.023 (0.020–0.026)
Dust	6 (4.6%)	99 (19.6%)	0.000 (0.000–0.000)
Soil	4 (3.1%)	82 (16.2%)	0.000 (0.000–0.000)
Faeces	4 (3.1%)	78 (15.4%)	0.000 (0.000–0.000)
Digestive tract	1 (0.8%)	2 (0.4%)	1.000 (1.000–1.000)
Dairy	2 (1.5%)	10 (2.0%)	1.000 (1.000–1.000)
Symptoms			
Fever	0 (0.0%)	394 (78.0%)	0.000 (0.000–0.000)
Sweating	0 (0.0%)	209 (41.3%)	0.000 (0.000–0.000)
Fatigue	0 (0.0%)	191 (37.7%)	0.000 (0.000–0.000)
Headache	0 (0.0%)	132 (26.1%)	0.000 (0.000–0.000)
Knee ache	0 (0.0%)	68 (13.4%)	0.000 (0.000–0.000)
Backache	0 (0.0%)	111 (21.9%)	0.000 (0.000–0.000)
Arthralgia	0 (0.0%)	114 (22.5%)	0.000 (0.000–0.000)
Testicular pain	0 (0.0%)	21 (4.2%)	0.022 (0.019–0.025)
Muscle pain	0 (0.0%)	17 (3.4%)	0.058 (0.054–0.063)
Shoulder ache	0 (0.0%)	21 (4.2%)	0.022 (0.019–0.025)

We also compared the manner of contact between participants with asymptomatic infection and those with symptomatic infection. It is known that the main means of contact are skin, fur, secretions and carcasses [13]. Interestingly, there were no significant differences between the two groups regarding contact with skin, fur, dairy or abortion-related products. However, patients with symptomatic infection had higher contact frequencies with contaminated soil and animal faeces (Table 1). As *Brucella* exists in high quantities in these environments [14], the higher frequency of contact with these substances may have resulted in symptomatic infection. This is instructive for prevention of *Brucella* infection: in addition to avoiding conventional risk factors, high-risk persons should also avoid contact with soil, faeces and dust contaminated by animals.

In this study, we confirmed that asymptomatic brucellosis infection occurs in some individuals. Asymptomatic infection may result from contact with animals harbouring lower-virulence species of *Brucella* or from less frequent contact with contaminated soil, faeces and dust. Symptomatic infections were due to frequent contact with contaminated faeces, soil and dust, where the pathogen persists for a long period [10]. Highly frequent exposure to these contaminated environments might lead to higher infection load of *Brucella* and cause the symptomatic infection. Identification of individuals with asymptomatic brucellosis is important for its early diagnosis, prevention of chronic infection, and tracking of disease prevalence. Awareness of the different clinical symptoms found in symptomatic infections is important for early diagnosis. Individuals in endemic areas who have a history of contact with potentially infected animals and their products and environments should be alerted to the risk of asymptomatic *Brucella* infection in humans when there is a sudden increase in incidence [15]. Asymptomatic infection is of great

value for early diagnosis of brucellosis in non-endemic areas. Clinicians should take into consideration the exposure of individual patients and the possibility of asymptomatic infection with *Brucella*.

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Transparency Declarations

The authors have no conflicts of interest to declare.

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